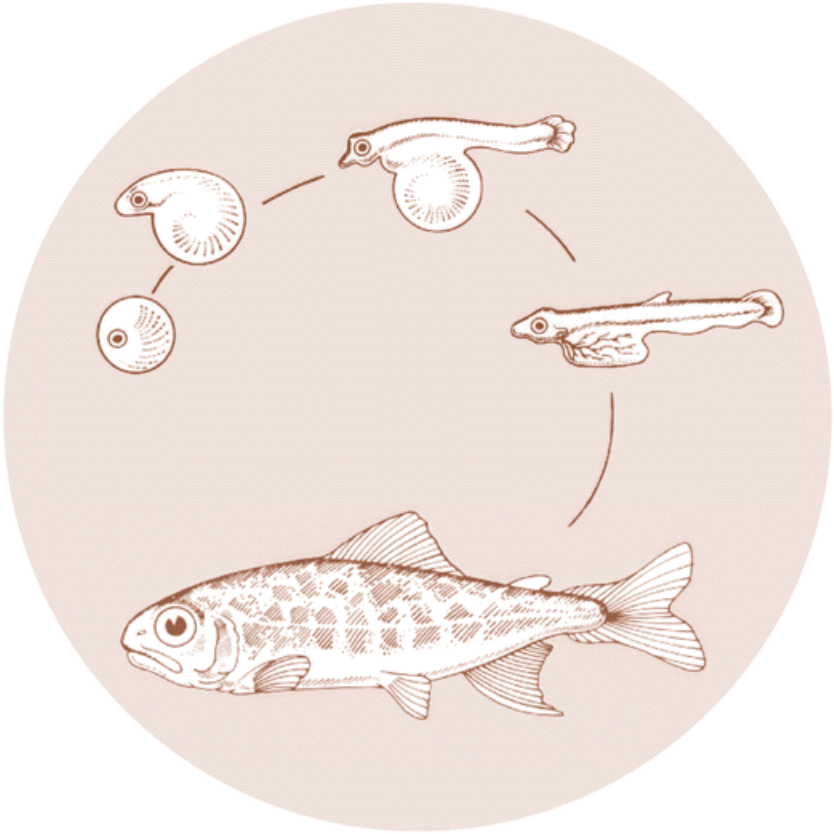
April 1991

FDA APPROVED REGISTRATION OF ERYTHROMYCIN FOR TREATMENT OF BACTERIAL KIDNEY DISEASE (BKD) IN JUVENILE AND ADULT CHINOOK SALMON

Reporting Period:
March 10, 1989 to March 9, 1990

Annual Report



DOE/BP-96247-1

This report was funded by the Bonneville Power Administration (BPA), U.S. Department of Energy, as part of BPA's program to protect, mitigate, and enhance fish and wildlife affected by the development and operation of hydroelectric facilities on the Columbia River and its tributaries. The views of this report are the author's and do not necessarily represent the views of BPA.

This document should be cited as follows:

Christine A. Moffitt, University of Idaho - Dept. of Fish and Wildlife Resources, FDA Approved Registration of Erythromycin for Treatment of Bacterial Kidney Disease (BKD) in Juvenile and Adult Chinook Salmon, 1991, Annual Report, Reporting Period: March 10, 1989 to March 9, 1990 to Bonneville Power Administration, Portland, OR, Contract 89PS96247, Project 89-32, 46 electronic pages (BPA Report DOE/BP-96247-1)

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FDA APPROVED REGISTRATION OF ERYTHROMYCIN FOR TREATMENT OF BACTERIAL KIDNEY DISEASE (BKD) IN JUVENILE AND ADULT CHINOOK SALMON

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March 10, 1989 to March 9, 1990

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Project No. 89-032
Contract No. DE-PS79-89BP96247

April 1991

ABSTRACT

Erythromycin is a therapeutic substance useful against bacterial kidney disease in salmon. In 1989 we began a multi year project to learn more about erythromycin applied to juvenile and adult salmon, with the goal of achieving registration of erythromycin with the U. S. Food and Drug Administration. To begin the study, we studied the pharmacokinetics of erythromycin administered to both adult and juvenile chinook salmon. We monitored blood plasma time curves from individual adult fish injected with two forms of injectable erythromycin using one of three routes of administration. In addition, we began experiments to evaluate hatchery applications of erythromycin to individually marked adult salmon, and we recovered blood and tissues from these fish at the time of spawning. To determine how to use erythromycin in juvenile salmon, we evaluated the absorption and elimination of erythromycin applied arterially and orally to individual juvenile fish. In feeding trials we determined the palatability to juvenile chinook salmon of feed made with one of two different carriers for erythromycin thiocyanate.

INTRODUCTION

Erythromycin is used in salmon culture to treat bacterial kidney disease (BKD) caused by *Renibacterium salmoninarum* (Wolf and Dunbar 1959; Groman and Klontz 1983; Austin 1985; Herman and Bullock 1986; Elliott et al. 1989; Moffitt and Bjornn 1989; Moffitt in press 1991); however, the correct dosages and durations or frequency of treatment for adult and juvenile salmon have not been determined. Erythromycin is not presently a therapeutic substance registered with the U.S. Food and Drug Administration, and all applications are conducted using Investigational New Animal Drug (INAD) permits.

The purpose of this project is to obtain the data necessary and conduct the studies needed to register erythromycin for use against BKD in salmon. The project has a 6 year duration with multiple objectives, many of which 'run concurrently. This is the first annual report.

SIGNIFICANT ACCOMPLISHMTS BY OBJECTIVE

Objective 1. Collect background data on the use of erythromycin as an injectable and orally administered drug, including data on specific uses in salmonid fish to reduce mortality due to bacterial kidney disease.

Background data on erythromycin must be assembled as part of the drug registration package. We have conducted an extensive review of the literature about erythronlycin with the assistance of Dr. Daniel Baker of the Drug Information Center at Washington State University. Erythromycin is a macrolide antibiotic isolated from *Streptomyces erythreus* (Gilman et al. 1985). It is most stable above pH of 6.0 to approximately 8.0, and decomposition occurs rapidly as pH decreases to 4.0 (Johnston 1982). In veterinary and human applications, erythromycin is an effective antimicrobial substance used to treat infections caused by most of the gram-positive bacteria, with limited usefulness in staphylococcal and gram negative injections. Since the majority of bacterial pathogens affecting salmonids are gram negative, the application of erythromycin therapy for salmon culture has been limited to *Renibacterium salmoninarum.*

The antibacterial mode of action of erythromycin is through attachment to the 50 S subunit of the bacterial ribosome. Although the exact stage of protein synthesis affected by erythromycin is unknown, erythromycin may interfere with the translocation reaction, most likely binding to the donor site, preventing translocation of the peptide chain (Kucers and Bennett 1979; Kastrup 1990). Depending on the concentration of drug, organism susceptibility, growth rate of the organism, and size of inoculum, erythromycin may have bacteriostatic or bactericidal properties

(Gilman et al. 1985). The antibacterial activity of erythromycin is enhanced in an alkaline environment, and activity increases progressively over pH range 5.5 to 8.5, since the nonionized form of drug is more permeable to cells (Gilman et al. 1985; AMA 1986). Since the physiological pH of salmonids is near to or equal to the optimum pH, the action of erythromycin is particularly effective.

Forms of erythromycin available for use In fish

Since no registered forms of erythromycin are available for use in fish culture, present applications utilize drug formulated for other veterinary usages. Most of the erythromycin that is used in North America is manufactured by Abbott Laboratories, North Chicago, Illinois, but Abbot has signed agreements with Sanofi Animal Health (formerly CEVA Laboratories) giving Sanofi the rights to distribute all veterinary usage of erythromycin in North America. As a result most of the products available have a trademark from Abbott Laboratories, and a usage label printed by Sanofi Animal Health.

Injectable erythromycin- The form of erythromycin available as veterinary injectable is erythromycin base, sold as Erythro-100 or Erythro-200, formulated in polyethylene glycol 400, ethyl acetate, and ethyl alcohol. This product is stable at room temperature with a shelf life of over 2 years. As formulated, it is either 100 or 200 mg/mL active drug (Erythro-100 or Erythro-200, respectively). In addition, some fish health personnel use aqueous solutions of erythromycin phosphate that they Prepare themselves from the dry salt, ranging in concentration from 40 to

50 mg/mL active drug, since the phosphate salt is only about 6% soluble in water.

Oral erythromycin - Most applications of erythromycin in fish feed have utilized commercially available Gallimycin 50 poultry formula, a mixture of erythromycin thiocyanate salt with a coarse corn carrier at 11% activity, or 50 g per pound of carrier. Erythromycin thiocyanate is insoluble in water. The carrier is mixed with the feed dough and pelleted to the appropriately sized pellet for feeding. A limited number of operations, particularly in British Columbia, use a powder of erythromycin phosphate, applied on top of pelleted feed that is covered with oil to absorb the powder.

Studies of efficacy against Renibacterium salmoninarum

Adult salmon - Injection of erythromycin into pre spawning adult salmon has been shown by some researchers to reduce vertical transmission of BKD (Groman and Klontz 1983; Evelyn et al. 1986; Armstrong et al. 1989; Brown et al. 1990a,b). In many hatchery operations fish health personnel inject erythromycin into the "dorsal sinus," a sub cutaneous region surrounding the dorsal fin, of maturing pre-spawning salmon. Dosages are generally 10 - 22 mg/kg, and are applied once to three times before spawning (Moffitt et al. 1990). The mechanism of reduction of vertical transmission of BKD infection may be due to residual drug retained in the ova and developing embryos from adult fish injected with erythromycin. T. P. E. Evelyn and his colleagues have studied the pharmacokinetics of erythromycin in the salmon egg and determined that the drug is sequestered in the maturing ova

after one injection of 20 mg/kg in the dorsal sinus of a pre spawning female salmon (Evelyn et al. 1986; Armstrong et al. 1989). Erythromycin is present in the eggs for some time post injection, and may last in the fertilized embryo until alevin stage in some circumstances. Bullock and Leek (1986) found erythromycin present in 70 d sac fry from adults injected twice before spawning with 11 mg/kg.

Juvenile salmon - Oral administration of erythromycin to juvenile salmon generally decreases mortality from BKD; however, several researchers have questioned the long-term effectiveness of the applications (Fryer and Sanders 1981; Herman and Bullock 1986; Elliott et al. 1989). Even though applications are considered experimental and supported by INAD permits, the use of erythromycin in the culture of juvenile Pacific salmon is common. The drug is used against outbreaks of BKD, and in some hatcheries prophylactic applications are administered during the rearing program. The most commonly applied oral dosage is 100 mg erythromycin per kg body weight per day administered over 21 consecutive days; however, some administrations are for 14 d, followed by a brief withdrawal, and then an additional 7 d of medicated ration (Moffitt et al. 1990). Few studies have been conducted to examine the effects of water temperature, dosages, and duration of treatment on therapeutic efficacy.

Moffitt and Bjornn (1989) studied the duration of protection against an acute challenge of *R. salmoninarum* following oral administration of 100 mg/kg daily dosage for 21 d. Protection against BKD was nearly complete when bacterial challenge followed the drug treatment, but was only partially effective when the challenge was administered 11 d later.

Recent work funded by the IR-4 program (Moffitt, in review) was conducted to investigate the efficacy of daily dosages of 50, 100 or 200 mg/kg erythromycin, applied for durations of 7, 14 or 21 d following a challenge with *R. salmoninarum* Each of the 9 drug treatments was applied to duplicate tanks of 50 yearling chinook salmon for each temperature (8 and 12°C) tested. In addition, tanks containing challenged fish not receiving drug were observed as controls. Fish fed rations containing the highest concentrations of erythromycin for a duration of 21 d had higher survival in tests conducted at both water temperatures. At the completion of the tests, the fish survived in tanks administered the highest dosages of erythromycin for 21 d had fewer clinical signs of BKD than did fish surviving in tanks of fish fed lower drug dosages and shorter durations. Within each level of drug dosage, increasing the duration of feeding resulted in increased survival.

Since BKD is considered ubiquitous throughout stocks of Pacific Salmon, dose titration experiments are compromised by the lack of disease free stocks of fish, and comparisons with control fish are increasingly important to any study. With the enzyme-linked immunosorbent assay (ELISA; Pascho et al. 1987) we can detect low levels of antigen from *R. salmoninarum* to allow a more precise prediction of the health of fish surviving a dose titration.

Objective 2. Validate all analytical methods to be used in the study, and determine the facilities and experimental animals that will be used.

Assay for erythromycin

Several scientists describe the use of an electrochemical (EC) detector for detection of erythromycin as it elutes from a high performance liquid chromatography (HPLC) column (Chen and Chiou 1983; Duthu 1984; Croteau et al 1987; Haataja and Kokkonen 1988). The use of HPLC with EC detection was considered the best alternative for analysis of erythromycin in this research project since it combined high sensitivity with specificity for unchanged erythromycin. HPLC using ultraviolet detection was determined to be relatively less sensitive, as was a microbiological analysis.

The following hardware were assembled in the laboratory of collaborator W. L. Hayton, Department of Pharmacology, Washington State University, to implement the published assay:

AT computer clone
Beckman System Gold Software (system controller, data analysis, data storage and retrieval)
EG&G PARC model 400 electrochemical detector.
Beckman HPLC pumps

By the end of July 1989, it was apparent that the EG&G detector would not respond to erythromycin. Using ^{14}-erythromycin and detection of radioactivity as it eluted from the column, we were able to establish chromatographic conditions for erythromycin that gave a retention time of six minutes. However, the detector would not oxidize erythromycin. The factory representative from EG&G, Ronald V. Wong, spent a day in the lab working on the problem and he, too, concluded that the detector was not

responding to erythromycin. The detector was responsive to other substances known to be detectable electrochemically and a malfunction of the detector was thereby ruled out. Dr. James Schenk, an analytical chemist on the faculty of WSU Department of Chemistry who specializes in electrochemistry and EC detectors, also worked with us to overcome the erythromycin detection problem. Using electrodes in his lab as well as our electrode, and a variety of solvent systems, no oxidation of erythromycin was produced. We cannot explain why the EG&G detector was insensitive to erythromycin.

During October and November 1989, we tried the HPLC method using ultraviolet detection erythromycin (Stubbs et al. 1985; Cachet et al. 1987; Tara et al. 1987). After this brief exploration period we decided that a successful assay was not possible within the time frame that we needed results, and we converted all our assay work to the microbiological method to determine concentrations of erythromycin in tissues, plasma, eggs and effluents.

For several years, the principal investigator used a microbiological assay (Moffitt and Schreck 1988) to determine μg quantities of erythromycin in plasma, tissues, and fish feed. In this method, the diameter of the zone of inhibition of 10 μL of an unknown substance applied to a paper disc is measured and related to a standard curve established for 10 μL quantities of four different known concentrations of erythromycin, prepared from standards and also applied to the discs. The linear regression least squares technique regressing the log of the concentration of erythromycin (y) versus the diameter of the zone of inhibition (x). Using this, we can

detect concentrations of erythromycin less than 1 μg of erythromycin. To obtain a more sensitive assay, we modified procedures in protocols established by the U.S. Food and Drug Administration (FDA) for erythromycin residues in poultry. The FDA method used a stainless steel disk template or discs to hold 200 μL of sample on top of an agar plate . seeded with an erythromycin sensitive microorganism, *Micrococcusluteus*. Since the steel disks were no longer commercially available, we removed cores from the seeded agar and filled this core with tissue or feed extract, plasma, or any aqueous sample. For flexibility of assay, we have cores for 100 and 200 μL. Using this method, we quantified the amount of erythromycin in the plasma samples removed from cannulated fish that were administered erythromycin during the Summer of 1989 that gave no detectable reading when assayed using the previous, less sensitive disc method. This modification allows us to detect concentrations ≤ 0.15 μg/mL erythromycin.

Hatchery and laboratory facilities

We requested permission from the Columbia Basin Fish and Wildlife Authority and the appropriate state, tribal or federal agencies for fish and facilities to use for our studies. This is an ongoing process and we will continue this communication each year we use experimental animals. In addition, the principal investigator continued to work with the University of Idaho to develop the designs for construction of the new wet laboratory that will be used for the adult work. The construction of the lab was originally scheduled for completion in April 1990, but delays at

the University caused us to postpone the adult work until 1991, when the lab was anticipated to be completed.

Standardize ELISA and FA techniques

We plan to evaluate the extent of infection of *R. salmoninarum* using the ELISA assay (Pascho et al. 1987) and standard florescent antibody tests (Bullock et al 1979). We are using the commercially available (Kirkegaard and Perry, Inc.) reagents for the ELISA and FA tests, and are using the protocols for ELISA obtained from R. Pascho from NFRC-S. We designed an experiment to test blind samples prepared in common at the University of Idaho in a four-way comparison of ELISA techniques. We made arrangements with Stephen Kaattari (Oregon State University), Diane Elliott, and Ronald Pascho (National Fishery Research Center, Seattle (NFRC-S) and Joe Lientz of Dworshak National Fish Health Center to assay a set of identical samples that we would also assay in our laboratory. We prepared the kidney homogenates to be used in the tests and made arrangements for these assays to be conducted in the summer of 1990. In addition this test will allow us to compare our direct FAT procedures and results on replicate slides prepared from the same homogenate with results from NFRC-S and Dworshak FHC.

Objective 3. Work with the appropriate pharmaceutical company in conjunction with USFWS, NFRL La Crosse, WI to develop a suitable formulation for injectable and oral administration of erythromycin.

We established contact with Drs. Fred Meyer, Bill Gingerich, and Rosalie Schnick, USFWS, NFRL La Crosse, WI. We communicate regularly about the progress of our work. Ms. Schnick is in contact with many researchers

working in chemical registration for aquaculture, and has been very helpful throughout the project.

The communications with CEVA (SANOFI) laboratories are handled through Mr. Urban Wessling, and Dr. Myron Brown. We send them quarterly reports and they have made drug available to use for all phases of the study. They have expressed reservations about marketing any new form of injectable drug, but they do seem willing to provide erythromycin thiocyanate in a wheat carrier, rather than the coarse corn base that is normally produced. We are working with their formulator, Lowell Macy about details of products.

Objective 3A. Select the drug form for adult injection.

Pharmacokinetics and bioavailability of erythromycin in adult salmon

Few studies have determined the pharmacokinetics of erythromycin in salmon. To define an appropriate dosage regime for treatment, we need a model of how the drug is absorbed and eliminated after administration in both juvenile and adult fish. Because pre spawning adult salmon move from marine to fresh water environment and do not feed for up to 6 months before spawning, it is very important to address the pharmacokinetics in maturing adult salmon. Models from the mammalian literature are useful for comparison, but they may in fact provide little information applicable to drug physiology in poikilotherms with such diverse life cycles.

Adult chinook salmon from Eagle Creek National Fish Hatchery and from Cowlitz Salmon Hatchery were used for studies of pharmacokinetics at the

University of Idaho fisheries wet laboratory in 1989. Fish for the experiments were obtained 17 May, 16 June, and 14 July. The fish were brought to the University of Idaho fisheries wet laboratory in a refrigerated 1,200 gallon fish truck. Upon arrival at the laboratory, the fish were anesthetized, weighed, measured and tagged with a uniquely numbered jaw tag. Fish were then placed into a large holding flume until experimentation, maintained at temperatures of 10 to 13'C. Single fish were transferred to circular tanks for acclimation to 10°C before experimentation.

The cannulation procedure is as follows: fish are transferred from a circular tank to a large cooler containing water with 100 mg/L MS222, and an air stone delivering oxygen. After the fish is anesthetized, it is transferred to a surgical table, ventral side up and the gills are irrigated with oxygenated water containing 75 mg/L MS222. The mouth is secured open with a hook. Two sutures are placed on the dorsal surface of the oral cavity, and a small hole is made through the tip of the snout and sutures applied to the top of the snout. Using properly sized I.V. catheter, the dorsal aorta is penetrated by pushing the catheter inward at an 30' angle at a location midway between the junction of the second gill arches with the dorsal aorta. When blood appears in the flash tube of the catheter, tubing is placed into the aorta and the sutures are tied to keep the tubing in the roof of the mouth. The tubing is fed through the hole in the snout, and a syringe with heparinized Ringer's solution is placed on the end of the cannula. Following placement of the cannula. the gills

are irrigated with water without anesthesia, until the fish begins to recover.

After several unsuccessful attempts to allow the cannulated adult salmon to swim freely in a 1.2 m-diameter, circular tank, we elected to place them in a plexiglass box that confines the fish, yet supplies a flow of water throughout. We placed 5 to 6 boxes into a 3.6 m long, 500 L trough maintained with single-pass temperature-controlled water at 10 °C (Figure 1).

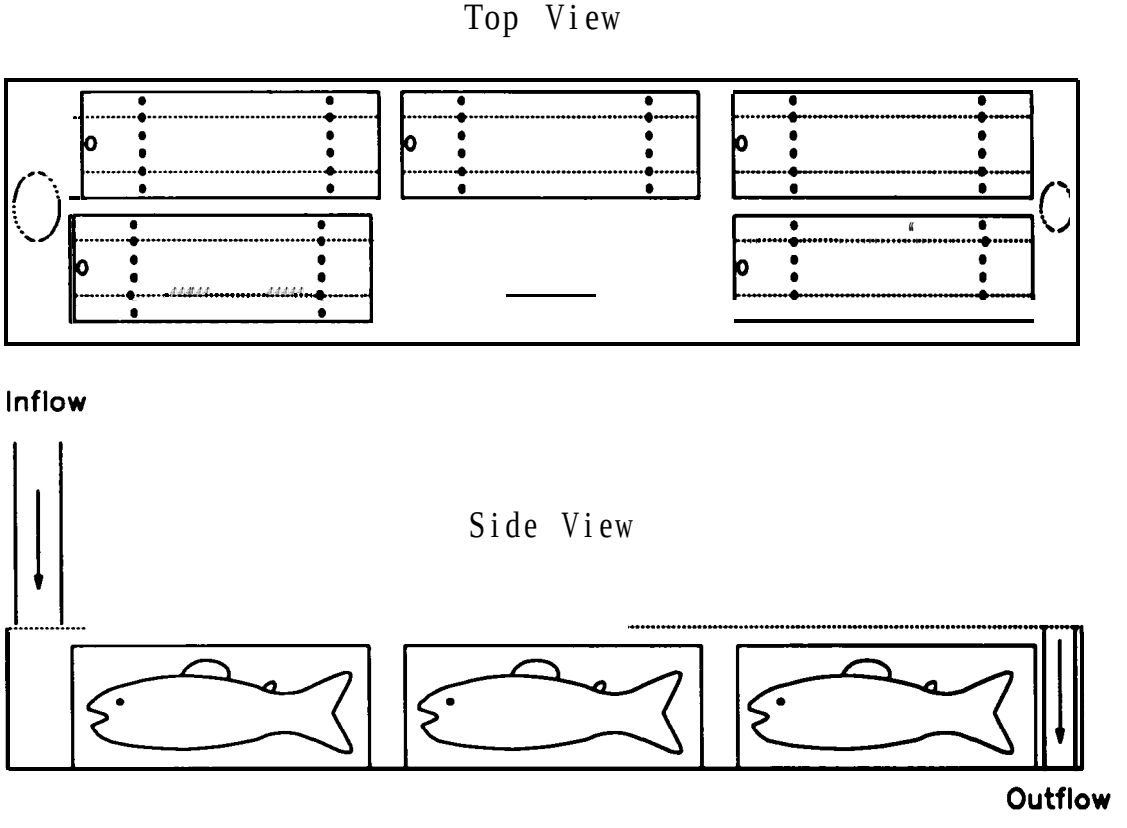

Figure 1. Schematic diagram of plexiglass boxes with cannulated adult salmon used for pharmacokinetics studies in University of Idaho wet lab.

Erythromycin was applied into the aorta directly through the cannula, and following administration we collected samples of blood from the fish.

After the first few experiments, we determined that the length of time for collection of blood samples needed to be extended because erythromycin remained for some time in the fish following a single administration. We established the sampling protocol to remove blood samples 0.5, 1, 2, 4, 8, 14, 24, 35, 48, 72, 120, 168, and 240 h following administration. These were nominal times for sampling; actual sampling times were recorded and used in the modeling. By using a 10 d observation period, we could quantify most portions of drug absorption and depletion. At each sampling interval, we collected approximately 1 mL of blood in a heparinized syringe and transferred this to a heparinized micro centrifuge tube and spun it to separate the red blood cells. The plasma fraction was stored frozen (-20') until analysis.

We conducted experiments between 17 May and 22 August 1989, to follow the absorption and elimination of erythromycin in the plasma of cannulated adult salmon held at 10°C, following a single dose of erythromycin phosphate or erythromycin base administered in the dorsal aorta (IA), the dorsal sinus (DS), the peritoneal cavity (IP), or the musculature (IM) (Table 1).

Table 1. Summary of administration of erythromycin to individual adult chinook salmon, University of Idaho wet lab, 1989.

Form of erythromycin	Dosage mg/Kg	Route of administration	Number of fish sampled
Base (early fish)	100	IA	4
	75	IA	4
	10	IA	5
	10	IP	12
	10	DS	6
	10	IM	2
Base (late fish)	10	IA	8
	50	IA	1
Phosphate (early)	50	IA	6
	10	IA	4
	10	IP	11
	10	DS	8
	10	IM	1

The plasma samples from each administration were analyzed for erythromycin content and individual plasma time profiles were constructed for each fish. For fish with complete and consistent profiles, model parameters were determined using the statistical moment technique. The statistical moment method approach involves the following calculations:

1. The plasma elimination half-life of erythromycin ($t_{\frac{1}{2}\beta}$). The plasma concentrations from 48 h post injection to t7e time of the last sample (t') was plotted on semi logarithmic coordinates. In the fish studied, these data fell along a straight line, the slope of which was determined by linear regression; the absolute value of this slope is referred to as "beta". ($t_{\frac{1}{2}\beta}$) was calculated from $\ln 2/\beta$. A minimum of three points had to be present on the log-linear part of the semilog plot to permit this analysis.

2. Area under the plasma concentration-time curve (AUC).
   a. The trapezoidal method was used to compute the area from time equal zero to the time of the last sample, t'.
   b. The area beyond the last sample was computed as C'/β, where C' is the last measured plasma concentration.
   c. The AUC was determined as the sum of the calculated and extrapolated areas.

3. The area under the first moment of the plasma concentration-time curve (AUMC).
   a. The trapezoidal method was used to compute the area under the first moment curve ($C \cdot t$ vs. t) from time equal zero to the time of the last sample.
   b. The area beyond the last sample was computed from:

$$AUMC(t' \text{ to infinity}) = (t' \cdot C'/\beta) + C'/\beta^2$$

   c. The AUMC was determined as the sum of the calculated and extrapolated areas.

4. The total body clearance of erythromycin (CL,,). CL, was calculated as the ratio dose/AUC.

5. The mean residence time (MRT) was calculated as the ratio AUMC/AUC.

6. The steady-state volume of distribution (V,,) was calculated as the product of $MRT \cdot CL_b$.

7. The volume of distribution during the beta phase ($V\beta$) was calculated as the ratio CL,, /β.

We found that erythromycin has a fairly long half-life following administration. Ten days after a single administration into the dorsal aorta of 75 mg aqueous erythromycin phosphate per kg body weight, detectable levels of drug were observed in the plasma of the adult salmon (Figure 2). Preliminary assessment of these data showed that plasma concentration time curves (AUC), appeared to be directly proportional to dosage, for both forms tested. While some differences in the kinetic parameters were observed at the different dosages, the differences did not appear large and did not occur in the total body clearance or the AUC, which would indicate that dosing rates are not complicated by non-linear kinetics. In addition, measurement of availability, or extent of absorption, can be based directly on AUC values, since AUC is directly proportional to dosage.

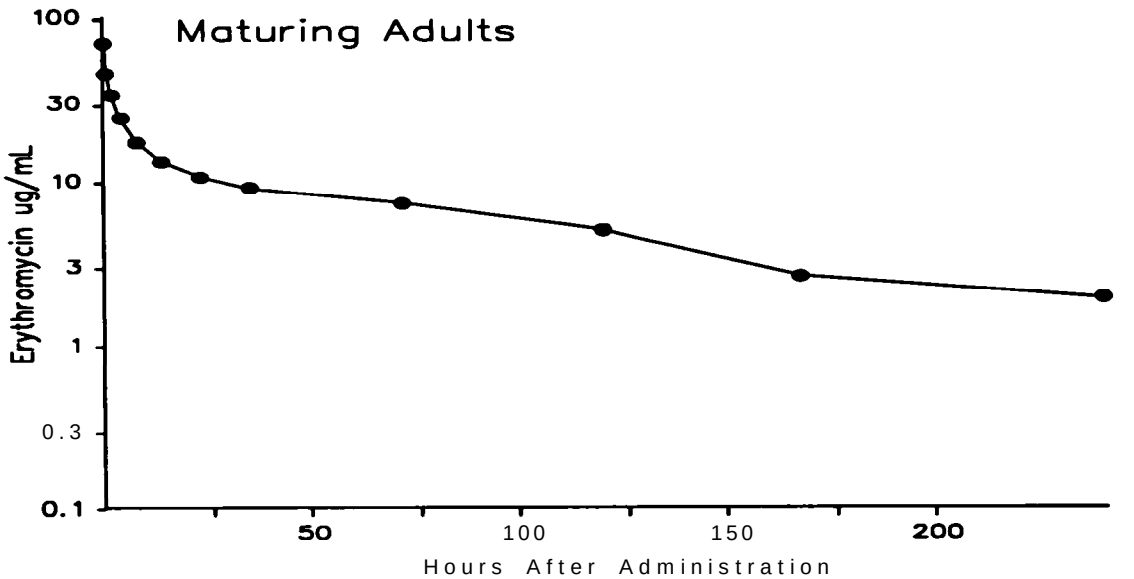

Figure 2. Concentration of erythromycin over time in plasma of individual adult spring chinook salmon following a single administration of 75 mg of aqueous erythromycin phosphate per kg body weight into the dorsal aorta. Drug administration and removal of blood samples were accomplished using a cannula placed in the dorsal aorta.

The pharmacokinetics of erythromycin administered intraarterially as a free base and as a phosphate salt were similar. We have considerable variation between fish, particularly in looking at the rate of absorption and elimination following IP, DS, or IM applications. Because the base is administered in an organic solvent, it was considered possible that there might have been a toxic effect of the vehicle on the erythromycin eliminating systems of the fish at a very high dose.

From our early estimations of body clearance the chinook salmon have total body clearance that is only about 5% of that observed in comparably sized mammals. The rate of absorption of erythromycin phosphate following administration appears faster than the absorption following administration of the base form in PEG 400. A faster absorption may be preferred and will allow for greater storage of erythromycin in the tissues, given the

apparent dose dependency of volume of distribution determined in our pharmacokinetic modeling. We have determined that both the base and phosphate salt are completely absorbed after IP administration, but after dorsal sinus administration only about half the administered dose may be absorbed, presumably because part of the dose leaks from the needle hole at the injection site. This leaking was observed following hatchery applications and in our laboratory. This phenomenon is also reported by other personnel at other hatcheries, but we had never realized the extent of the loss. We will include results from additional tests conducted in 1990 before making our final conclusions about the differences in pharmacokinetics. We will use Analysis of Variance techniques to detect significant differences in AUC between forms and routes at one dose.

Hatchery tests

To determine the compartmental distribution of drug in various tissues following administration to maturing adult salmon, we conducted injection experiments in 1989 on production spring chinook salmon at two hatcheries in the Columbia River drainage: Dworshak and Little White Salmon National Fish Hatcheries. Water temperatures at Dworshak ranged from 10 - 13°C and those at Little White Salmon were 8 - 10°C during the June to August holding period. Prior to injection, each fish was anesthetized with tricane (100 mg/L), weighed (0.5 kg), marked individually with a jaw tag, and injected in the dorsal sinus with approximately 10 mg erythromycin base (Erythro-200) per kg body weight. Injections were applied to different groups of fish at intervals prior to spawning, and at spawning: we collected samples of tissues and plasma from each tish, eggs and

ovarian fluid from females, and samples of testis from males for analysis of erythromycin content. The assay for erythromycin was conducted using the modified tissue extraction method described earlier. Erythromycin was detectable (>0.2 μg/g or mL) in many tissues and fluids of fish injected one to two weeks before spawning (Figure 3), and detectable levels of erythromycin remained in the kidney and eggs of injected fish for a longer period of time. The general profiles of concentration of tissues and fluids sampled at the two hatcheries were similar, but minor differences may be due to the differences in water temperatures, and in the injection process. At Dworshak the fish injected were part of general hatchery production, and were handled using mechanical crowders, with some fish not fully anesthetized when injected. The fish injected at Little White Salmon were fully anesthetized, and were handled separately from production fish at time of injection.

In addition to the hatchery production experiments, we were able to conduct tests of adult chinook salmon at Cowlitz hatchery in which we injected the fish at three different times before spawning with either 0, 20, and 40 mg/ kg erythromycin base, applied to the dorsal sinus. Fish were placed into experimental tanks at the hatchery, and each fish was individually marked with a jaw tag. We had fish of each of the three treatments in each tank. Most fish that were not injected with erythromycin died before spawning. In addition, we had unusually high mortality at time of spawning in all groups, probably because the fish were overripe. We collected tissues and fluids from fish at one time, but the remainder of the fish died and were never spawned. We will analyze the content of erythromycin in tissues collected from sampled fish.

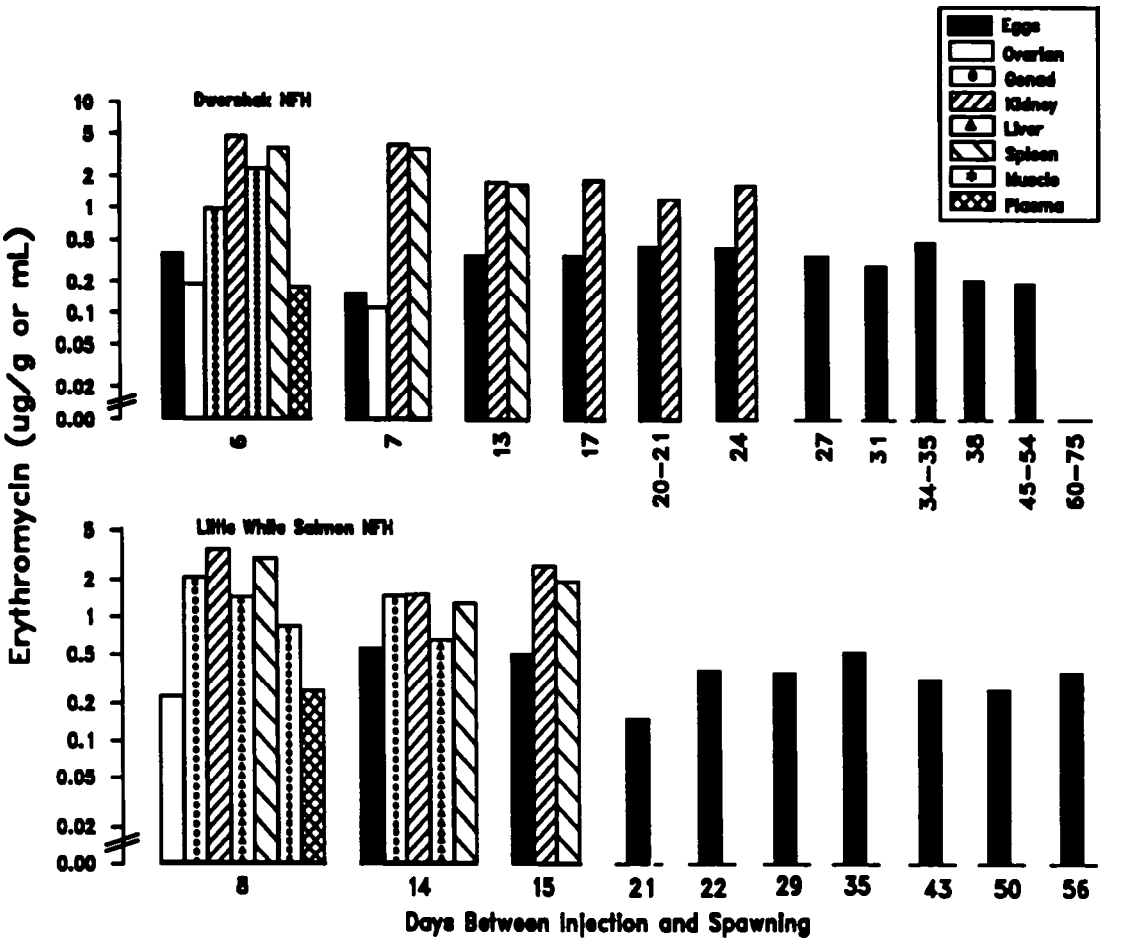

Figure 3. Erythromycin as μg/mL (plasma or ovarian fluid) and μg/g wet weight (liver, spleen, kidney, white muscle) from spawning adult spring chinook salmon injected once with 10 mg/kg eythromycin base as Erythro-200 into the dorsal sinus. The upper profile is from fish injected and spawned at Dworshak National Fish Hatchery (NFH); the lower profile is from fish injected and spawned at Little White Salmon National Fish Hatchery (NFH). Tissues were collected at the time of spawning, and days between injection and spawning are indicated below each profile along the horizontal axis. Data are means of samples of fluids or tissues from 1 - 14 fish that tested positive for erythromycin. At many sampling intervals, tissues and fluids from some fish tested positive for erythromycin, but tissues and fluids from others showed trace or no detectable drug residue. Within one tissue type, and hatchery location, when > 50% of the samples analyzed showed trace or no detectable residues of erythromycin, the values are not reported in the figure. For Dworshak NFH, sampling intervals with < 50% of samples containing no detectable erythromycin, but with some samples with detectable drug residues were: 7 d - gonad, liver, and plasma; 13 d - liver; 17 d -spleen; 20-21 d - spleen; 24 d - spleen; 27 d kidney. For samples from Little White Salmon NFH, intervals with < 50% of samples containing detectable concentrations of erythromycin, but some samples with detectable residues were: 8 d - eggs; 14-15 d - liver; 15 d ovarian fluid; 21-22 d - kidney; 22 d - spleen; 29 d - spleen.

In Vitro Tissue and Plasma Bindina;

To determine the extent of in vitro binding that occurs in blood and selected body tissues of adult salmon, the reversible binding of erythromycin to homogenates of selected tissues was determined using 222Spectrum teflon dialysis cells. Tissue homogenates from fresh and freshly frozen tissue samples were studied. ^{14}C-erythromycin was used to determine the concentration of erythromycin in each dialysis cell chamber. The ^{14}C was determined to be 76% radiochemically pure by thin layer chromatography using the protocols described by Kibwage et al. (1983). While a higher purity would have been preferred, the binding of erythromycin was quite low and it can be concluded that the figures reported below would not change appreciably if the non-erythromycin radioactivity bound to a greater or lesser extent than did the erythromycin (Table 2). The tissue homogenate was placed in one chamber of the dialysis cell and a buffer containing ^{14}C-erythromycin was placed in the other chamber. The cell was rotated in a 10°C water bath for at least 3 h, which preliminary experiments showed was sufficient for equilibrium.

Table 2. Extent of in vitro binding of ^{14}C-erythromycin from selected tissues from adult chinook salmon. Mean ± standard error are presented from three fish except for frozen tissue from anterior kidney (n = 2). [a] indicates the homogenate solidified (gelled) in the dialysis cell chamber, equilibrium was not achieved, and no value was determined.

Tissue	Percent bound	
	Fresh	Frozen
Posterior kidney	1.5 ± 2.6	2.3 ± 0.7
Anterior kidney	7.0 ± 2.0	3.3 ± 2.8
Liver	4.5 ± 1.6	5.4 ± 3.4
Spleen	2.2 ± 3.8	0.4 ± 0.5
Eggs	3.3 ± 3.0	[a]

Binding was very low for the tissues studied. No significant differences in the extent of binding of erythromycin in fresh versus freshly frozen tissues were observed. When these tissues are removed from live juvenile fish fed erythromycin (Moffitt and Schreck 1988), or from adults injected with erythromycin, the concentration of erythromycin in them is several times higher than that observed in the plasma. These in vitro binding studies suggest that the high and persistent tissue concentrations of erythromycin in vivo are not due to passive binding of erythromycin to the tissue components, but may be active processes. Preparation of the homogenates disrupts the cellular structure of the tissues and the in vitro binding studies do not reflect the capacity of the intact cells to accumulate erythromycin. A probable explanation of the in vitro vs. in vivo difference in erythromycin binding is that erythromycin is accumulated in intact cells by an active transport system that maintains the large concentration gradient. More in vitro studies using live, cultured cells would be useful. The stability of erythromycin for long periods of time in the unfertilized egg and in the vitellin of fertilized eggs indicates that some kind of stable binding occurred.

Objective 3B. Select drug formulation for juvenile feeding.

Pharmacokinetics and bioavailability of erythromycin in juvenile salmon

When erythromycin is added to the feed, the ability of the gut to absorb the drug determines how much will enter the fish. To test the rate and amount of absorption across the gut wall in juvenile chinook salmon, we first performed cannulation experiments.

Juvenile chinook salmon 100 g and larger were anesthetized with 100 mg/L tricane, placed ventral side up on a surgical table, and the jaw secured open with a hook similarly to the adult salmon. We irrigated the gills with oxygenated water containing 50 mg/L tricane using a hand held squeeze bottle placed beneath the operculum. We placed one suture on the dorsal surface of the oral cavity and one suture on the snout using a half circle size 20 suture needle. We made a small hole through the tip of the snout cartilage using a 19 or 21 gauge thin wall I.V. catheter. Then using a 19 or 21 gauge thin wall I.V. catheter we penetrated the dorsal aorta at approximately 45' angle at a location midway between the junction of the second gill arch. At the first sign of blood entering the flash tube of the catheter, the 30 gauge cannula tubing was pushed 4-5 cm into the aorta while simultaneously pulling out the catheter. We then threaded the 30 gauge tubing through the hole in the snout, and tied the sutures and placed a heparinized syringe at the end of the catheter. To be certain the cannula was in place, we lifted the syringe attached to the cannula to see if blood would enter. We then filled the cannula with heparinized Cortland ringers solution and irrigated the gills with oxygenated water without anesthetic until spontaneous jaw movement occurred. We then transferred these fish to 150 L circular tanks or 35 L individual aquaria and held them at 10°C. Fish were allowed to recover from the operation 20 to 24 h before administration of the drug.

To test pharmacokinetics of erythromycin in juvenile fish we administered a high and low dosage of erythrcmycin phosphate intra arterially through the cannula. Following injection, we removed serial blood samples from each at sampling intervals to characterize the distribution and

elimination stages: generally, 0.5, 1, 2, 4, 8, 14, 24, 48, 72, 120, 168, and 240 h post administration. Because of the limitation on the amount of blood that can be removed from a small fish, some fish were sampled only in the early phase, and some were sampled in the later phase. In some experiments where high dosage of drug was administered, we were able to remove small quantities of blood (0.1 cc) from one individual and use the disc method of assay (Moffitt and Schreck 1988) to determine erythromycin content.

As with the adult fish, we plotted the plasma time profiles for individual fish and then calculated pharmacokinetic parameters. From the basic profiles of intra arterial administration, we assume complete absorption and can established a profile of absorption and elimination. The bioavailability (F) or percentage absorbed, after oral application, will be calculated as the AUC oral / AUC IA adjusted for dose.

We determined that rate of absorption and elimination of drug was more rapid in the juvenile fish than maturing adult salmon. The concentration of erythromycin was below detectable levels in samples of plasma collected 7 d following administration of erythromycin phosphate (75 mg/kg body weight) into the dorsal aorta of cannulated juvenile chinook salmon (200 - 300 g)(Figure 4).

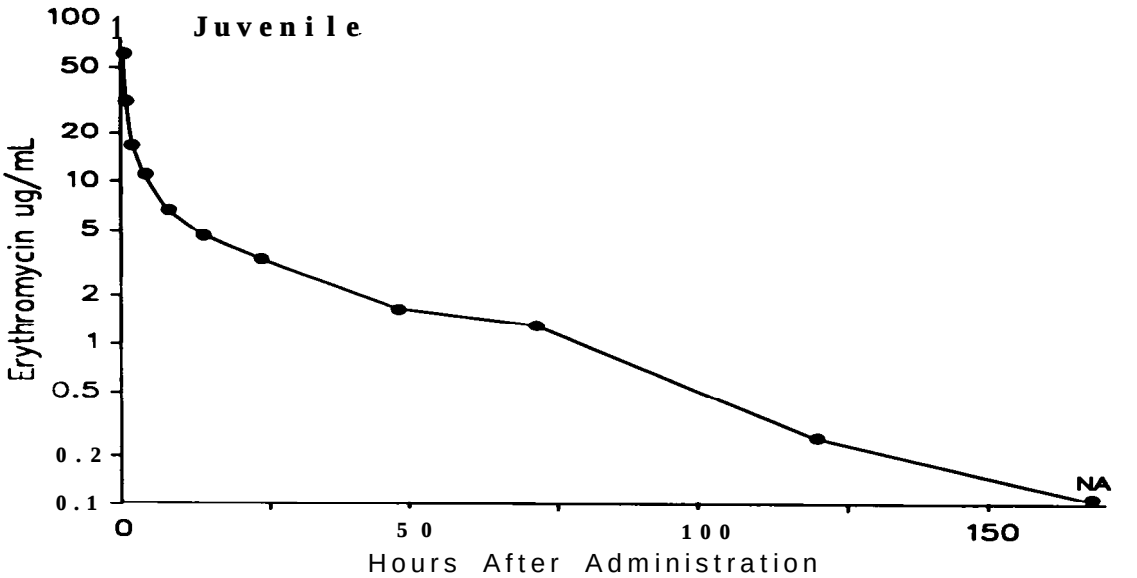

Figure 4. Concentration of erythromycin over time in plasma of individual uvenile spring chinook salmon following a single administration of 75 mg of aqueous erythromycin phosphate per kg body weight into the dorsal aorta. Drug administration and removal of blood samples were accomplished using a cannula placed in the dorsal aorta.

In addition, we collected tissues from fish administered drug, and will analyze these for drug content. We will determine the amount of material stored in tissues after dosing and calculate the total body loading of erythromycin. Evidence from previous experiments showed that concentration of erythromycin accumulated in tissues of fish administered oral dosages of 100 and 50 mg/kg of erythromycin per day for 21 d (Moffitt and Schreck 1988).

New carrier for erythromycin thiocyanate feed additive

The presently used carrier, Gallimycin 50 is 11% erythromycin and the remainder of the product is a ccarse corn based carrier. This carrier presents problems when preparing medicated feed that must be pelleted into a small pellet, and necessitates that the feed manufacturer grinds the carrier to · fine consistency before mixing. We proposed a new carrier

for the drug that is finely ground wheat flour that can be pelleted into small sized feed, and also contains twice the concentration of active drug (22% vs 11%). SANOFI indicated that they could produce this product with little problem.

Palatability of feed

The palatability of feed is important to the efficacy of oral treatments. If a fish refuses to eat the medicated ration the drug treatment can not be effective. Past studies determined an increasing rejection of feed with increasing drug concentrations (Schreck and Moffitt 1987).
In July, 1989 we mixed 13 batches of feed (six with the old carrier and six with the new carrier and one control, non-medicated diet), using the facilities at the Oregon State University Seafoods Laboratory at Astoria, Oregon, in cooperation with Dr. Dennis Roley, nutritionist at BioProducts. We brought this feed back to the University of Idaho laboratory, and stored it frozen (-20°C) until it was used in the feeding trials. We also tested the concentration of erythromycin in single pellets, and in batches of pellets to confirm the target amounts of drug.

From July 1989 to May 1990, we tested the palatability of this feed to groups and to individual yearling chinook salmon, brood year 1988 from Dworshak National Fish Hatchery. Each trial of groups of juvenile salmon was designed to test palatability to fish of feed at one drug concentration made with either the old Gallimycin,, or the new Gallimycin,,. The palatability of groups of fish was tested at two temperatures: 15 and 10°C. Concentrations of drug in the feed were

adjusted for each temperature tested to yield a target daily drug consumption of 50, 100 or 100 mg/kg body weight. Because of limited facilities, tests were conducted sequentially for each dosage and temperature tested. For trials conducted at 15 and 10°C, we assumed a feeding rate of 1.8% and 1% body weight per day, respectively, and manufactured the diets accordingly to provide the three dosages (Table 3).

Table 3. Summary of all test diets used in palatability tests. Diet code is expressed in parentheses beside percentage of carrier, and daily dosage is calculated assuming feeding at 1.8 and 1.0% body weight per day.

----------Percent---------- Gallimycin 50	Gallimycin 100	Erythromycin ug/mg	Daily dose mg/kg
	Trials at 15°C		
2.53 (A)	1.26 (D)	2.8	
5.05 (B)	2.53 (E)	5.6	1x8
10.1 (C)	5.05 (F)	11.1	200
	Trials at 10°C		
4.54 (G)	2.25 (J)	5.0	50
9.10 (H)	4.54 (K)	10.0	100
18.18 (I)	9.09 (L)	20:o	200
Control (M)		0.0	0

Appropriately sized fish were selected for the tests by selecting fish 10 mm either side of a mode length. For all trials fish ranged from 105 to 160 mm total length. A total of nine 100 L rectangular glass aquaria were stocked with 15 fish each. Fish in three aquaria were administered a ration made with either Gallimycin,,, Gallimycin,,, or the control ration. After the fish acclimated to the aquarium, a feeding trial was conducted tor 21 d. The percent of target ration consumed in each tank was

evaluated daily, and summarized weekly. A mean weight gain was determined for each aquarium, and expressed as a percent gain per fish.

The target daily ration for each aquarium was weighed (0.1 g) into labeled plastic cups, and half of it offered between 0800-0900 h and the remainder between 1600 to 1700 h. We administered feed to each aquarium by dropping pellets of feed into each aquarium using a spoon. Feeding of fish in the aquarium was terminated when five pellets dropped to the bottom and were not consumed in one minute. If the fish in an aquarium refused to eat when the feed was first offered, we returned to that tank and repeated the feeding procedure after all other aquaria were tested. Feed remaining in each cup after each feeding was weighed (0.1 g) and recorded. Tanks were cleaned at the end of each day by removing the standpipe to drain no more than half volume. Once each week, sides and bottoms were cleaned. Fish that died during the experiment were weighed, measured and examined for signs of BKD. If the trial was in its first 7 d, the fish was replaced with a weighed and measured fish. After 7 days the loss was not replaced. At the beginning and the end of each trial, all fish were weighed and measured. Fish were used in one test only.

The experimental design fits a split plot design with repeated measures and tanks nested in treatments.

Model: $Y_{ijk} = U + A_i + Bj(i) + CK + A*Cik + E_{ijk}$, Where:

U = Overall mean

A_i =Treatments (i = 1, 2, 3), where 1 = control; 2 = diet with Galli 50, and 3 = diet with Galli 100

$B_{j(i)}$ = jth tank nested in ith treatment (j = 1, 2, 3).

C_K = Week (k = 1, 2, 3)

$A*C_{ik}$ = Interaction between weeks and treatment

E_{ijk} = Error

From preliminary analysis of our results, and the percentage of the target feed consumed decreased as the concentration of erythromycin in the feed increased. At 15°C with administration of a diet containing 11.1 mg/g erythromycin, the fish lost weight and consumed only one third of the target diet, compared to a 15% gain in weight and consumption of 94% of the daily target ration for fish fed the control diet. In tests using 2.8 mg/g erythromycin in the feed, fish fed a diet made with the new carrier, Gallimycin,,,, accepted the ration more readily than did fish fed a ration made with the old carrier, Gallimycin,, (96 vs 91.1% of target). These differences may be due to the fact that feed manufactured with the new carrier has more ration per pellet, since the portion of the new carrier needs to be only half that used for the old. These differences in growth or acceptance of the pellets were not observed feeding rations with a higher drug content. The new carrier may not improve palatability significantly, but there were no adverse effects, and it may give better growth.

Tests of palatability to individual fish

We tested more than 400 fish in individual palatability trials at 15°C. Fish previously acclimated to living in glass aquaria for at least 3 d, were placed alone in one side of a small aquarium and allowed to acclimate for at least one day before testing. On the day of the test, only fish

that accept an initial pellet of Biodiet 3.0 mm feed without erythromycin just before testing were used. These fish have never had any prior experience with feed containing erythromycin. Individual fish that accepted the non-test diet were then offered a single pellet of feed of one of the 7 diets (A - F, and M), chosen at random. The following aspects of the behavior of the fish are observed and recorded: the time to strike the pellet, the number of ejections per pellet, whether the pellet is consumed or not, and the time to consume the pellet. A second pellet of the same diet was offered to each test fish at least five minutes after the first, and the same behavioral responses were recorded.

From a preliminarily summary of the tests completed, fewer fish ate the second pellet of feed containing drug, following administration of a pellet of feed containing drug. These differences were consistent regardless of concentration of drug in the pellet or carrier. Other parameters recorded have shown considerable variation between fish. We are continuing to analyze these data.

Techniques to overcome palatability

Spray coating is often used in the drug industry to overcome palatability problems with a drug. Historically, erythromycin thiocyanate feed grade used in Gallimycin,, was spray coated with methyl cellulose. However, the feed manufacturers quit this process in 1987, and now the feed is not coated at all. During 1989, we made preliminary plans for trying a spray coating technique on a test batch of drug. We identified some of the different possibilities of coating the erythromycin with a film to

increase palatability of feed mixed with the drug. Film coating involves the deposition of a thin, uniform membrane onto the surface of a substrate. There are three major components in the film coating of drugs: film forming resin (polymer), a solvent, and a plasticizer. The most commonly used film formers are the cellulose ethers. In July, we met with Dr. Mark Christianson, professor of Pharmacy at Oregon State University, about using some of the equipment in their laboratory to produce test batches of feed. Dr. Christianson recommended the use of aquacoat as a film forming resin. According to Christianson, it is composed of ethylcellulose and is the cheapest to use, easiest to get, and easiest to use. However, Porter et al. (1982) and Ellis et al. (1976) write that ethylcellulose is not suitable for use by itself, but should be used with other cellulose ethers. Christianson states that there is no advantage to mixing ethylcellulose with hydroxypropyl methylcellulose (HPMC) or other cellulose ethers, and that doing so may cause the feed to pass completely through the fish without ever releasing the coated drug.

We plan to prepare rations with spray coated drug and with non-coated drug and test the palatability to groups of juvenile chinook salmon of this feed.

Objective 4. Conduct laboratory and field studies to develop data to support a registration application.

Preoaration of protocols and Good Laboratorv Practices

We prepared the protocols and experimental design for juvenile and adult dose titration experiments to submit to FDA. The review will be accomplished during the second year of the project.

To submit a drug registration package, data must be collected using good laboratory practices, or GLP's. Furthermore, tests of dose titration must be conducted using protocols that have been previously evaluated and approved by FDA. We began to follow Good Laboratory Practice regulations in all our experimental research. The principal investigator handed out copies of these requirements to all investigators and reviewed these requirements with examples. We initiated GLP's for experimental work in both the wet and dry laboratory. We have protocols for all procedures on file and store all data in a locked metal filing cabinet. All samples are stored in a locked freezer. Access is limited in areas of research.

The progress on the following objectives will not be reported as activity on these portions of the project is scheduled for years 2-5.

Objective 5. Determine the minimum inhibitory concentration of the chosen injectable and dietary forms of ● rythranycin against the grwth of KD bacteria (KDB) at two different incubation temperatures.

Objective 6. Determine the optimal dosage and frequency of administration of injectable erythromycin to reduce mortality from a challenge of bacterial kidney disease in adult chinook salmon.

Dbjectivc 7. lest at least three drug dosages, and 3 treatment durations to determine the optimal dosage and duration of treatment for the ● rythroycin thiocyanate form of choice (Task 3.1) in treating BKD in juvenile chinook salmon at two different water temperatures.

Objective 8. Establish the safety limits of the optimal dosages determined for adult broodstock (injectio $ CA' and for juveniles (by feeding: 88) in Objective 7, by determining the toxicity of dosages of 1, 3, and 5 X the target dosage and 3X the duration.

Objective 9. Determine the tissue residues of ● rythroycin in chinook salmon tilt broodstock and in chinook salmon juveniles at appropriate times during and following administration of the selected optimal dosage.

Objective 10. Conduct necessary clinical field trials to establish the treatments as viable rthods in hatchery applications.

Objective 10A. Conduct clinical field trials on selected stocks of adult chinook salmon at approved sites to demonstrate efficacy and safety of the injection of erythromycin to prevent or control BKD.

Objective 10B. Conduct clinical field trials on selected stocks of juvenile chinook salmon at approved sites to demonstrate efficacy and safety of the feeding of erythromycin to prevent or control BKD.

Objective 11. Determine and model the environmental fate of erythromycin as it will be delivered to adult or juvenile chinook salmon in normal fish cultural use patterns.

Objective 12. Develop registration data package and submission to FDA.

Objective 13. Prepare and submit completion report to Bonneville Power Administration that is suitable for publication in a peer-reviewed journsl.

EVALUATION OF WORK SCHEDULE

All components of the work schedule are progressing on schedule with the exception of the delay in beginning the adult dose titration tests. These tests were scheduled to begin in spring of 1990, but more time was needed to evaluate the pharmacokinetics of administration of erythromycin to adult salmon, and the new laboratory that the University of Idaho is building for these tests was not completed. This delay gives us more time to evaluate the differences between injections applied to the peritoneal cavity versus those administered into the dorsal sinus. Additionally, the extra year allowed us to expand the number of individually cannulated fish administered erythromycin of different dosages and routes of administration. With these changes the completion of the adult dose titration tests will not occur until 1992, and testing of toxicity will begin in 1992. We anticipate that these changes will not alter the completion date for the project, but just for these elements.

PROPOSED WORK FOR PROJECT YEAR 2, 1990-91

Objective 1. Collect background data on the use of ● rythrcmycin as an injectable md orally administered drug, including data on specific uses in salmonid fish to reduce mortality due to bacterial kidney disease.

Work will continue keeping the background data up to date, and preparation for the drug registration package. We plan to supplement this search with information obtained from a survey of hatchery use of erythromycin, and assess what we know of information on the environmental effects of applications or manufacturing of erythromycin.

Objective 2. Validate all analytical methods to be used in the study, and determine the facilities and experimental animals that will be used.

We plan to continue validating all analytical methods. We will complete the multi-way ELISA and FAT comparison of blind samples of homogenated kidney tissue.

objective 3. Work uith the appropriate pharmaceutical company in conjunction with USFWS, RFRL La Crosse, WI to develop a suitable formulation for injectable and oral administration of erythromycin.

Objective 3A. Select the drug form for adult injectim.

We plan to continue our work with pharmacokinetics of erythromycin administered to adult salmon so that we can select the most appropriate form for injection. This activity will be completed during the next year.

Objective 3B. Select drug formulation for juvenile feeding.

We will continue with our evaluations of the bioavailability of erythromycin administered orally to chinook salmon, and determine if there are any disadvantages to a new carrier.

Objective 4. Conduct laboratory and field studies to develop data to support a registration application.

All our studies are conducted using Good Laboratory Practices. We will continue to instruct all workers about the need for these in all aspects of the study.

Objective 5. Determine the ● iniu inhibitory concentration of the chosen injectable and dietary forms of erythromycin against the growth of KD bacteria (KDB) at two different incubation temperatures.

We plan to begin to address this objective in the next year.

Objective 6. Determine the optimal dosage and frequency of administration of injectable ● rythroqcin to reduce mortality from a challenge of bacterial kidney disease in adult chinook salmon.

We will submit our protocols to FDA for review in the coming year, and plan to begin these trials in the third project year, 1991.

Objective 7. lest at least three drug dosages, and 3 treatment durations to determine the optimal dosage and duration of treatment for the erythromycin thiocyanate form of choice (Task 3.1) in treating BKD in juvenile chinook salmon at two different water temperatures.

We will submit our protocols to FDA for review in the coming year, and plan to begin trials late in 1990.

PROPOSED WORK FOR FUTURE PROJECT YEARS

Objective 8. Establish the safety limits of the optimal dosages determined for adult broodstock (injection: 9A) and for juveniles (by feeding: 88) in Objective 7, by determining the toxicity of dosages of 1, 3, and 5 X the target dosage and 3X the duration.

We will begin planning for this objective in year 3, but do not plan to begin tests until year 4.

Objective 9. Determine the tissue residues of ● rythromycin in chinook salmon tilt broodstock and in chinook salmon juveniles at appropriate times bring and following administration of the selected optimal dosage.

We will submit protocols for these tests during year 3 of the project.

Objective 10. Conduct necessary clinical field trials to establish the treatments as viable methods in hatchery applications.

Objective 10A. Conduct clinical field trials on selected stocks of adult chinook salmon at approved sites to demonstrate efficacy and safety of the injection of ● rythraaycin to prevent or control BKD.

Limited clinical field trials will be conducted during year 3, and plans for more trials made for year 4.

Objective 10B. Conduct clinical fielc trials on selected stocks of juvenile chinook salmon at approved sites to demonstrate efficacy and safety of the feeding of ● rythrorycin to p event or control BKD.

Clinical field trials will be planned during year 3, and implemented in year 4.

Objective 11. Determine ad model the environmental fate of ● rythrauycin as it will be delivered to adult or juvenile chinook salmon in normal fish cultural use patterns.

Planning will begin during year 3, activity during year 4.

Objective 12. Develop registration data package and s&mission to FDA.

Portions of the registration package will be assembled throughout the project, with the final portions put together during years 5 and 6 of the project.

Objective 13. Prepare and submit completion report to Bonneville Power Administration that is suitable for publication in a peer-reviewed journal.

We plan to publish throughout the duration of the project, and the final completion report will be the major activity of the 6th year.

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PERSONNEL YEAR 1

Scientists

Christine M. Moffitt: (U of I) Principal Investigator and lead scientist of project, responsible for overall coordination, experimental design, logistics and reporting.

Expertise in microbiological assay for erythromycin, tissue residues of erythromycin in juveniles, hatchery injection protocols, erythromycin feeding trials, efficacy of erythromycin against bacterial kidney disease, and diagnostics and culture of bacterial kidney disease.

Theodore C. Bjornn:(U of I). Co-investigator, Fish Biology.

Expertise in experimental design, hatchery logistics, feeding trials, efficacy of erythromycin against bacterial kidney disease.

William Hayton, (WSU) Co-investigator, Pharmacology.

Expertise in pharmacokinetic modeling of drugs and other compounds administered to fish and other vertebrates.

Research Associates and Laboratory Technicians

Gwynne L. Chandler- (Research Associate U of I) Fish biology

Analytical and wet laboratory coordination, data analysis and computer graphics. Hatchery injections and tissue sampling.

Cheryl Hughett (Laboratory Technician, U of I). Microbiology

Analytical laboratory erythromycin assay

Janine Nesheim (Laboratory Technician, U of I). Microbiology

Analytical laboratory erythromycin assay, ELISA, FAT, Culture of *Renibacterium salmoninarum*

Rudy R. Ringe (Research Associate, U of I). Fish Biology

Wet laboratory logistics and fish life support systems. Transportation of adult and juvenile fish. Hatchery injection, spawning and tissue sampling.

Graduate Research Assistants and Major Responsibilities:

Colleen E. Fagan: U of I. Fish biology.

Palatability and feeding of erythromycin, kinetics of erythromycin in juveniles.

Irvin R. Schultz: USU. Pharmacology.

Drug kinetics in adults.

Student Laboratory and Field Assistants:

WSU

Jim Meyer: HPLC assay for erythromycin, tissue binding study.
Rudy Kucera: Drug kinetic sampling and laboratory assistance.

u of I

Lorraine Blasch: Care of fish, wet laboratory support
Matthew Campbell: Care of fish, laboratory support, hatchery sampling.
Mariah Clark: Care of fish, laboratory support, hatchery sampling.
Sonia Eby: Laboratory support
Todd Gammill: Laboratory support
Kara Olson: Laboratory support, hatchery sampling, data entry.
Marc Petersen: Care of fish, hatchery sampling.
Donna Prisbrey: Care of fish, data entry, hatchery sampling.
Christine Wells: Laboratory support
David Williams: Care of fish, laboratory support
Chow Shing Uong: Microbiological analysis for erythromycin.

Additional support:

Dan Baker: USU Drug information Center. Background information on safety and environmental problems associated with erythromycin.